BACTERICIDAL TREATMENT OF HATCHING EGGS III: EFFECT OF ORGANIC CONTAMINANTS ON EFFICACY OF EGG SANITIZERS

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Primary Audience: Hatchery Managers, Quality Control Personnel, Plant Managers

SUMMARY

The effectiveness of three sanitizers in killing *Salmonella* during room temperature storage with and without contaminating feces, egg, or chicken rinse for 48 h was tested. Uncontaminated sanitizers were tested, as were 50-mL solutions of 0.035% polyhexamethylenebiguanide hydrochloride (PHMB), 0.39% (1:256) Tektrol, and 1.4% H_2O_2, which were contaminated with 10 mL of a feces slurry, 10 mL of mixed egg, or 10 mL of chicken rinse. Each container was inoculated with approximately 1,000 cells of *S. typhimurium* and cultured after 1 and 5 min. After 24 and 48 h of storage, each original container was reinoculated and assayed for the new inoculum at 1 and 5 min postinoculation. Complete eradication of *Salmonella* was noted after 1 or 5 min exposure to fresh or stored uncontaminated sanitizer solutions. Tektrol became less active against *Salmonella* when contaminated with egg and stored for 24 h or more. However, feces or chicken rinse did not interfere with the activity of Tektrol. Hydrogen peroxide deteriorated most with storage with chicken feces and to a lesser extent chicken rinse. PHMB lost efficacy when exposed to chicken feces for 24 h or more, but was not affected by the other contaminants.

Key words: Hatching eggs, *Salmonella*, sanitizers

2001 J. Appl. Poult. Res. 10:117–120

DESCRIPTION OF PROBLEM

The need to disinfect broiler hatching eggs was recognized at least as early as 1908 when Pernot [1] reported on the use of formaldehyde gas to control microbial populations. Since the Occupational Safety and Health Administration (OSHA) published its lists of concerns [2] on the effects of repeated or prolonged exposure to formaldehyde, there have been several studies evaluating various chemical sprays or dips as alternatives to formaldehyde. Sheldon and Brake [3] and Padron [4] found hydrogen peroxide (H_2O_2) to be an effective eggshell sanitizer. Scott and co-workers [5, 6, 7] evaluated the effectiveness of 23 sanitizers against a cocktail of inoculated microorganisms. They found Germex, Io-

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cide-14, Lysovet, Quam, hydrogen peroxide, Quat 800, Quat 900, 1-Stroke (Tektrol has similar ingredients), and Virkon to meet their criteria and warrant large-scale hatchability field trial studies.

In our laboratory at the Russell Research Center, Cox and co-workers [8, 9] have evaluated 16 different commercial hatching egg sanitizers using an egg spray sanitizing machine. Polyhexamethylenebiguanide hydrochloride (PHMB) at 0.035%, H_2O_2 at 1.4%, and Tektrol at 0.39% were found to be the most effective for killing *Salmonella* on and beneath the eggshell surface. None of these extensive research projects has evaluated the effect of "normal" environmental contaminants on the effectiveness of nongaseous chemical sanitizers. Commercial hatching egg spray sanitizing machines may use recycled sanitizing solutions. The chemical is sprayed on eggs, collected, and used again. Recycling of chemicals helps to hold down the cost of sanitization but may lead to contamination of the wash liquid. In addition these chemicals have been proposed as possible sprays or chiller water additives to kill salmonellae and other bacteria on processed broiler carcasses. The purpose of the present study was to determine the short- and long-term effects of the organic contaminants chicken feces, chicken rinse fluid, and whole broken eggs on the effectiveness of PHMB, H_2O_2, and Tektrol as sanitizers to kill *Salmonella*.

Materials and Methods

SALMONELLA CULTURE

A nalidixic acid-resistant strain of *Salmonella typhimurium* was used for all experiments. This culture was maintained frozen at −70°C in 10% glycerol. Inocula were grown overnight on BG sulfa agar [10] with the addition of 200 ppm sodium salt of nalidixic acid [11] (BGS+NAL). Cells were suspended in 0.85% NaCl; the optical density was measured with a spectrophotometer, and the number of cells for the inoculum was determined by using a standard curve. Inoculum level was confirmed by serial dilution and plate count (24 h, 35°C) on BGS+NAL.

EGG SANITIZERS

Three chemicals at concentrations previously shown to be effective at eliminating *Salmonella* from eggshells [8, 9] were tested for stability: PHMB at 0.035%; H_2O_2 at 1.4%; and Tektrol at 0.39% (1:256 or 1 oz/gallon). All chemicals were diluted in sterile distilled water. Sanitizers were prepared in duplicate 50-mL volumes using sealable sterile sample cups.

EXTRANEOUS ENVIRONMENTAL MATERIALS

Each sanitizer was tested through time with the addition of environmental contaminants. We tested plain sanitizer with no added material; sanitizer mixed with 10 mL autoclaved chicken rinse, obtained by shaking a carcass in a bag with 100 mL sterile water [12]; sanitizer mixed with 10 mL of autoclaved Leghorn hen fecal slurry (one part feces and one part water); and sanitizer with 10 mL fresh whole mixed egg.

SAMPLE CULTURE

Within 10 min after the addition of the extraneous organic material, duplicate cups were inoculated with approximately 1,000 cells of nalidixic acid-resistant *Salmonella*. At sampling times, each cup was throughly mixed, and a 0.1-mL sample was removed with a sterile pipette. The sample was added to 9 mL of buffered peptone (BP) with 5% milk to inactivate any viable chemical present. After overnight incubation at 35°C, a sterile cotton swab was moistened in the BP and used to apply the sample to the surface of a BGS+NAL plate. Plates were incubated overnight at 35°C and read as positive or negative for *Salmonella* presence. Isolates were confirmed as *Salmonella* with *Salmonella* Poly O and *Salmonella* Poly H serology.

Samples were drawn from each cup at 1 and 5 min postinoculation. Sanitizers were then allowed to remain uncovered at room temperature for 24 h to simulate storage on a farm. After the 24 h storage, each cup was re-inoculated and resampled at 1 and 5 min postinoculation. This procedure was repeated again after 48 h sanitizer storage.

Results and Discussion

Inoculated, unadulterated eggshell sanitizer samples were consistently negative for *Salmonella* before and after 24 to 48 h of storage. Furthermore, contaminants did not reduce the

TABLE 1. Recovery of *Salmonella*[A] after 1 and 5 min exposure to chemical sanitizers in the presence of organic contaminants for 0, 24, and 48 h

	0 h STORAGE				24 h STORAGE				48 h STORAGE			
	1 min		5 min		1 min		5 min		1 min		5 min	
SANITIZER[B]/CONTAMINANT	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2
PHMB	−	−	−	−	−	−	−	−	−	−	−	−
Chicken rinse	−	−	−	−	−	−	−	−	−	−	−	−
Chicken feces	−	−	−	−	+	−	+	+	−	−	+	+
Egg contents	−	−	−	−	−	−	−	−	−	−	−	−
Tektrol	−	−	−	−	−	−	−	−	−	−	−	−
Chicken rinse	−	−	−	−	−	−	−	−	−	−	−	−
Chicken feces	−	−	−	−	−	−	−	−	−	−	−	−
Egg contents	−	−	−	−	+	+	+	+	+	+	+	+
H_2O_2	−	−	−	−	−	−	−	−	−	−	−	−
Chicken rinse	−	−	+	−	−	−	+	−	+	−	−	−
Chicken feces	−	−	+	+	+	+	+	+	+	+	+	+
Egg contents	−	−	−	−	−	−	−	−	−	−	−	−

[A]Inoculum contained approximately 1,000 cells.
[B]PHMB = 0.035% polyhexamethylenebiguanide hydrochloride; 1:256 mixture of Tektrol, a commercial blend of phenols; 1.4% H_2O_2.

effectiveness of unstored sanitizers when tested for *Salmonella* after 1 min of exposure. Interestingly, increasing the H_2O_2 exposure time to 5 min led to recovery of *Salmonella* in some samples. This recovery is likely explained by the organic loads having partially inactivated the chemicals, which led to a reduction, but not a total kill, of the *Salmonella*. Therefore, it was a matter of whether or not the aliquot sampled had a viable *Salmonella* present.

The contaminants used in this study did not reduce the efficacy of PHMB or Tektrol when the inoculum was exposed for 5 min (Table 1). However, chicken rinse and feces interfered with the action of unstabilized H_2O_2, and the inoculated *S. typhimurium* were not killed. After 24 and 48 h exposure to the contaminants (Table 1), PHMB was inactivated only by chicken feces, Tektrol was inactivated only by egg contents, and H_2O_2 was inactivated primarily by feces and twice by chicken rinse.

The levels of contaminants used in this study would be considered high and were meant to reflect a worst-case scenario. When chemicals were not inactivated by the contaminants, it could be assumed that the chemicals would not be inactivated in a commercial setting where contamination rates would likely be lower. The methods used in this experiment were aimed at recovering even injured *Salmonella* cells. Therefore, the sanitizers were tested for the ability to completely eradicate *Salmonella*, not merely reduce the population. The effectiveness of Tektrol in killing *Salmonella* when no contamination was present was similar to that previously observed in our laboratory [8]. However, because of the possibility that eggs may crack or break and contaminate sanitizing solutions, the ineffectiveness of Tektrol after 24 and 48 h exposure to egg contamination would likely preclude this chemical from being used to routinely disinfect eggs unless wash solutions were changed when any egg contamination was observed. Egg contamination did not affect PHMB or H_2O_2, but feces did reduce the effectiveness of these chemicals. The buildup of fecal material washed from eggs being sanitized is almost inevitable in a machine that recycles sprayed chemicals and would likely begin to reduce the effectiveness of the PHMB or unstablized H_2O_2. Based on the results from this study, to maximize efficacy of chemical sanitation of fertile hatching eggs, our recommendation would be to change sanitizer solutions at least daily and more often if heavy organic loads are found in the sanitizing solution.

Conclusions and Applications

1. Storage of sanitizer solutions at room temperature for 24 to 48 h did not reduce their effectiveness in eliminating *Salmonella*.
2. The ability of Tektrol to kill *Salmonella* will be greatly reduced by exposure of the sanitizer to egg contents.
3. The ability of PHMB and H_2O_2 to kill *Salmonella* will be greatly reduced by exposure to feces.
4. Prior to egg sanitization, any cracked or broken eggs should be discarded to prevent contamination of sanitizer solutions.
5. Contaminated sanitizer solutions should be replaced with fresh solutions daily, or as needed.

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